

What is claimed is:

1. A recombinant multi-state genetic oscillator comprising:

(a) a first nucleic acid construct comprising a first inducible promoter operably associated with a first gene encoding a first protein;

(b) a second nucleic acid construct comprising a constitutive promoter operably associated with a second gene encoding a second protein and to a third gene encoding a third protein; and

(c) a third nucleic acid construct comprising a second inducible promoter operably associated with a fourth gene encoding a fourth protein, wherein

(i) the first protein, when produced, is capable of repressing transcription from the constitutive promoter,

(ii) the second protein, when produced, is capable of repressing transcription from the first inducible promoter,

(iii) the third protein, when produced, is capable of increasing transcription from the second inducible promoter, and

(iv) the fourth protein, when produced, is capable of increasing transcription from the first inducible promoter.

2. The genetic switch of claim 1, wherein the first and second proteins are DNA binding proteins capable of repressing transcription from a constitutive promoter.

3. The genetic switch of claim 1, wherein the first inducible promoter, the constitutive promoter or the second individual promoter is operably associated with an operator.

4. The genetic switch of claim 1, wherein the third and fourth proteins are DNA binding proteins capable of increasing transcription from an inducible promoter.

5. The genetic switch of claim 1, wherein the first construct further comprises a gene of interest in operable association with the first inducible promoter.

6. The genetic switch of claim 5, wherein expression of the gene of interest increases and decreases periodically.

5 7. The genetic switch of claim 1, wherein the second nucleic acid construct further comprises a gene of interest in operable association with the constitutive promoter.

8. The genetic switch of claim 7, wherein expression of the gene of interest increases and decreases periodically.

10 9. The genetic switch of claim 1, wherein the first and second nucleic acid constructs are disposed within a single contiguous nucleic acid molecule.

10. A host cell harboring the genetic switch of claim 1.

11. The host cell of claim 10, wherein the host cell is a prokaryotic cell.

12. The host cell of claim 10, wherein the host cell is a eukaryotic cell.

15 13. A method of periodically expressing a gene of interest in a host cell, the method comprising the steps of:

(I) providing a host cell harboring a recombinant oscillator comprising:

(a) a first nucleic acid construct comprising a first inducible promoter operably associated with a first gene encoding a first protein;

20 (b) a second nucleic acid construct comprising a constitutive promoter operably associated with a second gene encoding a second protein and to a third gene encoding a third protein;

(c) a third nucleic acid construct comprising a second inducible promoter operably associated with a fourth gene encoding a fourth protein, wherein

(i) the first nucleic acid construct, the second nucleic acid construct or both the first and second nucleic acid constructs further comprise a gene of interest,

(ii) the first protein, when produced, is capable of repressing transcription from the constitutive promoter,

(iii) the second protein, when produced, is capable of repressing transcription from the first inducible promoter,

(iv) the third protein, when produced, is capable of increasing transcription from the second inducible promoter, and

(v) the fourth protein, when produced, is capable of increasing transcription from the first inducible promoter; and

(II) growing the cells under conditions to permit periodic expression of the gene of interest.

14. The method of claim 13, wherein in step I, the first and second proteins are DNA binding proteins capable of repressing transcription from a constitutive promoter.

15. The method of claim 13, wherein in step I, the first inducible promoter, the constitutive promoter or the second inducible promoter is operably associated with an operator.

16. The method of claim 13, wherein in step I, the third and fourth proteins are DNA binding proteins capable of increasing transcription from an inducible promoter.

17. The method of claim 13, wherein in step I, the first and second nucleic acid constructs are disposed within a single contiguous nucleic acid molecule.

18. The method of claim 13, wherein in step I, the host cell is a prokaryotic cell.

19. The method of claim 13, wherein in step I, the host cell is a eukaryotic cell.

20. A recombinant multi-state genetic oscillator comprising:

(a) a first nucleic acid construct comprising an inducible promoter operably associated with a first gene encoding a first protein and a second gene encoding a second protein;

(b) a second nucleic acid construct comprising a first constitutive promoter operably associated with a third gene encoding a third protein; and

(c) a third nucleic acid construct comprising a second constitutive promoter operably associated with a fourth gene encoding a fourth protein, wherein

(i) the first protein, when produced, is capable of repressing transcription from the first constitutive promoter,

(ii) the second protein, when produced, is capable of repressing transcription from the second constitutive promoter,

(iii) the third protein, when produced, is capable of repressing transcription from the inducible promoter, and

(iv) the fourth protein, when produced, is capable of increasing transcription from the inducible promoter.

21. The genetic switch of claim 20, wherein the first, second and third proteins are DNA binding proteins capable of repressing transcription from a constitutive promoter.

22. The genetic switch of claim 20, wherein the inducible promoter, the first constitutive promoter, or second constitutive promoter is operably associated with an operator.

23. The genetic switch of claim 20, wherein the fourth protein is a DNA binding protein capable of increasing transcription from an inducible promoter.

24. The genetic switch of claim 20, wherein the first construct further comprises a gene of interest in operable association with the inducible promoter.

25. The genetic switch of claim 24, wherein expression of the gene of interest increases and decreases periodically.

- (ii) the first protein, when produced, is capable of repressing transcription from the first constitutive promoter,

(iii) the second protein, when produced, is capable of repressing transcription from the second constitutive promoter,

(iv) the third protein, when produced, is capable of repressing transcription from the inducible promoter, and

5 (v) the fourth protein, when produced, is capable of increasing transcription from the inducible promoter; and

(II) growing the cells under conditions to permit periodic expression of the gene of interest.

10 33. The method of claim 32, wherein in step I, the first, second and third proteins are DNA binding proteins capable of repressing transcription from a constitutive promoter.

34. The method of claim 32, wherein in step I, the inducible promoter, the first constitutive promoter, or the second constitutive promoter is operably associated with an operator.

35. The method of claim 32, wherein in step I, the fourth protein is a DNA binding protein capable of increasing transcription from an inducible promoter.

15 36. The method of claim 32, wherein in step I, the first and second nucleic acid constructs are disposed within a single contiguous nucleic acid molecule.

37. The method of claim 32, wherein in step I, the host cell is a prokaryotic cell.

38. The method of claim 32, wherein the host cell is a eukaryotic cell.

39. A recombinant multi-state genetic oscillator comprising:

20 (a) a first nucleic acid construct comprising an inducible promoter operably associated with a first gene encoding a first protein;

(b) a second nucleic acid construct comprising a first constitutive promoter operably associated with a second gene encoding a second protein; and

(c) a third nucleic acid construct comprising a second constitutive promoter operably associated with a third gene encoding a third protein, wherein

(i) the first protein, when produced, is capable of repressing transcription from the first and second constitutive promoters,

(ii) the second protein, when produced, is capable of repressing transcription from the inducible promoter,

(iii) the third protein, when produced, is capable of increasing transcription from the inducible promoter.

40. The genetic switch of claim 39, wherein the first and second proteins are DNA binding proteins capable of repressing transcription from a constitutive promoter.

41. The genetic switch of claim 39, wherein the first inducible promoter, the first constitutive promoter, or the second constitutive promoter is operably associated with an operator.

42. The genetic switch of claim 39, wherein the third protein is a DNA binding protein capable of increasing transcription from an inducible promoter.

43. The genetic switch of claim 39, wherein the first construct further comprises a gene of interest in operable association with the inducible promoter.

44. The genetic switch of claim 43, wherein expression of the gene of interest increases and decreases periodically.

45. The genetic switch of claim 39, wherein the second nucleic acid construct further comprises a gene of interest in operable association with the first constitutive promoter.

46. The genetic switch of claim 45, wherein expression of the gene of interest increases and decreases periodically.

47. The genetic switch of claim 39, wherein the first and second nucleic acid constructs are disposed within a single contiguous nucleic acid molecule.

48. A host cell harboring the genetic switch of claim 39.

49. The host cell of claim 48, wherein the host cell is a prokaryotic cell.

50. The host cell of claim 48, wherein the host cell is a eukaryotic cell.

51. A method of periodically expressing a gene of interest in a host cell, the method comprising the steps of:

(I) providing a host cell harboring a recombinant oscillator comprising:

(a) a first nucleic acid construct comprising an inducible promoter operably associated with a first gene encoding a first protein;

(b) a second nucleic acid construct comprising a first constitutive promoter operably associated with a second gene encoding a second protein; and

(c) a third nucleic acid construct comprising a second constitutive promoter operably associated with a third gene encoding a third protein, wherein

(i) the first nucleic acid construct, the second nucleic acid construct or both the first and second nucleic acid constructs further comprise a gene of interest,

(ii) the first protein, when produced, is capable of repressing transcription from the first and second constitutive promoters,

(iii) the second protein, when produced, is capable of repressing transcription from the inducible promoter, and

(iv) the third protein, when produced, is capable of increasing transcription from the inducible promoter; and

(II) growing the cells under conditions to permit periodic expression of the gene of interest.



52. The method of claim 51, wherein in step I, the first and second proteins are DNA binding proteins capable of repressing transcription from a constitutive promoter.

53. The method of claim 51, wherein in step I, the first inducible promoter, the first constitutive promoter, or the second constitutive promoter is operably associated with an operator.

54. The method of claim 51, wherein in step I, the third protein is an a DNA binding protein capable of increasing transcription from an inducible promoter.

55. The method of claim 51, wherein in step I, the first and second nucleic acid constructs are disposed within a single contiguous nucleic acid molecule.

56. The method of claim 51, wherein in step I, the host cell is a prokaryotic cell.

57. The method of claim 51, wherein in step I, the host cell is a eukaryotic cell.

58. A recombinant multi-state genetic oscillator comprising:

(a) a first nucleic acid construct comprising an inducible promoter operably associated with a first gene encoding a first protein;

(b) a second nucleic acid construct comprising a constitutive promoter operably associated with a second gene encoding a second protein; and

(c) a third nucleic acid construct comprising an inducible or constitutive promoter operably associated with a third gene encoding a third protein,

wherein (i) the first protein, when produced, is capable of repressing transcription from the constitutive promoter, (ii) the second protein, when produced, is capable of repressing transcription from the inducible promoter, and (iii) the third protein, when produced, is capable of increasing transcription from the inducible promoter, and

wherein (i) transcription from the first promoter increases when the amount of the third protein reaches a threshold level, and (ii) the amount of the third protein is under the control of a

regulatory gene product that is expressed from the promoter of the first construct or the promoter of the second construct.

59. The oscillator of claim 58, wherein the promoter of the third construct is an inducible promoter.

5 60. The oscillator of claim 59, wherein transcription from the inducible promoter of the third construct is increased by an expression product of the second construct

61. The oscillator of claim 58, wherein the promoter of the third construct is a constitutive promoter.

10 62. The oscillator of claim 61, wherein transcription from the constitutive promoter of the third construct is repressed by an expression product of the first construct.

63. The oscillator of claim 58, further comprising a gene of interest in operable association with the promoter of the first nucleic acid construct, the second nucleic acid construct or the third nucleic acid construct.

15 64. The oscillator of claim 58, wherein expression of the gene of interest increases and decreases periodically.

65. The oscillator of claim 58, wherein the regulatory gene product is the first protein or the second protein.

66. A host cell harboring the oscillator of claim 58.